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Multidisciplinary screening of toxicity induced by silica nanoparticles during sea urchin development

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HIGHLIGHTS

- Sea urchin fertilization ability was not affected by SiO_2 NPs exposure.
- A significant percentage of anomalies were observed and quantified in exposed samples.
- Altered expression of acetylated tubulin, ChAT and AChE was found in exposed samples.
- The multidisciplinary approach was able to verify SiO_2 NP effects in the offspring.

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ABSTRACT

The aim of this study was to investigate the potential toxicity of Silica nanoparticles (SiO_2 NPs) in seawater by using the sea urchin *Paracentrotus lividus* as biological model. SiO_2 NPs exposure effects were identified on the sperm of the sea urchin through a multidisciplinary approach, combining developmental biology, ecotoxicology, biochemistry, and microscopy analyses. The following responses were measured: (i) percentage of eggs fertilized by exposed sperm; (ii) percentage of anomalies and undeveloped embryos and larvae; (iii) enzyme activity alterations (acetylcholinesterase, AChE) in the early developmental stages, namely gastrula and pluteus. Sperms were exposed to seawater containing SiO_2 NPs suspensions ranging from 0.0001 mg/L to 50 mg/L. Fertilization ability was not affected at any concentration, whereas a significant percentage of anomalies in the offspring were observed and quantified by means of EC_{50} at gastrula stage, including undeveloped and anomalous embryos (EC_{50} = 0.06 mg/L), and at pluteus stage, including skeletal anomalies and delayed larvae (EC_{50} = 0.27 mg/L). Moreover, morphological anomalies were observed in larvae at pluteus stage, by immunolocalizing molecules involved in larval development and neurotoxicity effects – such as acetylated tubulin and choline acetyltransferase (ChAT) – and measuring AChE activity. Exposure of sea urchins to SiO_2 NPs caused neurotoxic damage and a decrease of AChE expression in a non-dose-dependent manner.

In conclusion, through the multidisciplinary approach used in this study SiO_2 NPs toxicity in sea urchin offspring could be assessed. Therefore, the measured responses are suitable for detecting embryo- and larval- toxicity induced by these NPs.

1. Introduction

Nanoparticles (NPs) are ultrafine particles with lengths in two or three dimensions greater than 1 nm and smaller than 100 nm (British Standards Institution, 2005; ASTM, 2006). Today, a broad spectrum of NPs of different chemical composition, size, shape and surface structure are commercially available with several industrial, biotechnological, and biomedical/pharmaceutical applications (Clèment et al., 2013). Among the various types of NPs, silica nanoparticles (SiO_2 NPs) have become popular as nanostructuring, drug delivery, and optical imaging agents (Yang et al., 2010). In particular, they are used in paints and coatings,

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attachment and scratch-resistance. In printer toners they serve as anti-binder (Mizutani et al., 2006; Zappa et al., 2009). In addition, these NPs are used in chemical or mechanical polishing processes, including dental polishing to prevent tooth caries (Gaikwad and Sokolov, 2008). Other medical applications use SiO_2 NPs as carriers of therapeutic agents or for diagnostic purposes (Wang et al., 2006; Zhang et al., 2008a). While their special physicochemical properties make them commercially attractive, SiO_2 NPs may pose potential hazards to human health and the environment due to their highly stable molecules, which could bioaccumulate in the environment (Nguyen et al., 2013). Since industrial products and domestic wastes tend to end up in waterways, the aquatic environment and biota are of a major concern (Moore, 2006; Baun et al., 2008). Therefore, any direct and/or indirect release of these NPs into the aquatic ecosystem may pose potential hazards to the environment. Indeed, several research works have fully demonstrated that SiO_2 NPs are toxic for freshwater and marine organisms (Van Hoecke et al., 2008; Canesi et al., 2010a,b; Falugi et al., 2012; Gambardella et al., 2013, 2014; Vo et al., 2014). SiO_2 NPs toxicity has already been reported for freshwater algae, namely *Pseudokirchneriella subcapitata*, *Chlorella kessleri* (Fujiwara et al., 2008; Van Hoecke et al., 2008) and for crustaceans, such as *Daphnia magna* (Lee et al., 2009). SiO_2 NPs have been reported to be stable in *P. subcapitata* test medium and that their ecotoxicity was related to their surface area. Further, size-dependent toxicity was reported for SiO_2 NPs in *C. kessleri* and dose-dependent toxicity in crustaceans (Fujiwara et al., 2008; Lee et al., 2009).

Toxicity of SiO_2 NPs has also been reported in the marine environment, caused by unwanted nanosized silica resulting from the release of commercial products, as well as from natural sources, such as coastal erosion, diatom deposits, via rivers to the coastal waters in front of some huge deposits (Buzea et al., 2007). Studies have proved that SiO_2 NPs may induce oxidative stress and evoke significant changes in lysosomal and enzymatic biomarkers in mussels at very high concentrations (up to 10 mg/L, Canesi et al., 2010a, b; Ciacci et al., 2012), apart from affecting algae growth (Zhang et al., 2008b). The uptake, transfer and toxicity associated to SiO_2 NPs from the marine microalgae *Cricosphaera elongata* to sea urchin *Paracentrotus lividus* larvae has recently been investigated. Our research indicates that SiO_2 NPs may enter the marine food chain and, as a consequence, cause abnormalities in developing sea urchin larvae. In addition, a significant survival percentage reduction was observed in sea urchin larvae fed with microalgae previously exposed to 1 mg/L SiO_2 NPs (Gambardella et al., 2014).

P. lividus is a valid model for ecotoxicology studies, and it has been recently proposed as a suitable organism for nanotoxicity studies. Indeed, toxicity tests have reported that its developmental stages are highly sensitive to several NPs (Fairbairn et al., 2011; Manno et al., 2012; Matranga and Corsi, 2012; Manzo et al., 2013; Siller et al., 2013). Due to their sensitivity and availability, *P. lividus* gametes are usually employed to assess toxicity of chemical compounds and NPs in particular. Therefore, spermiotoxicity tests may help study the effects evoked by NPs on developing sea urchin larvae. Sperm cells can also result in transmissible damage to the offspring when exposed to NPs (Gambardella et al., 2013; Manzo et al., 2013; Mesarič et al., 2015). However, there are no data yet about their toxic effects on *P. lividus* embryos and larvae from sperms exposed to SiO_2 NPs.

In this study, the effects of exposure to SiO_2 NPs were identified on the sperm of the sea urchin, through a multidisciplinary approach, combining developmental biology, ecotoxicology, biochemistry, and microscopy analyses. In particular, the following responses were measured: (i) percentage of fertilized eggs by exposed sperm; (ii) percentage of anomalies and undeveloped embryos and larvae; (iii) enzyme activity (acetylcholinesterase, AChE, E.C. 3.1.1.7) in the early developmental stages. Moreover, morphological anomalies were investigated in larvae at pluteus stage, by immunolocalizing molecules involved in neurotoxicity activity and larval development, such as acetylated tubulin and choline acetyltransferase (ChAT, E.C. 2.3.1.6).

2. Materials and methods

2.1. Nanoparticles

SiO_2 NPs with a nominal size between 4 and 40 nm were provided by Tal Materials Inc (USA) with at least 98% purity. NPs were characterized for size and effective surface charge (ζ-potential). Prior to each measurement, SiO_2 NPs were resuspended in ultrafiltered (0.22 μm Teflon filter) seawater (FSW, 37‰ salinity) for size characterization and in distilled water for measuring the effective surface charge at a concentration of 50 mg/L, and sonicated for 1 h using Branson 2510 bath sonicator (Branson Ultrasonic, Danbury, CT, USA). Size characterization of NPs dispersed in FSW was determined by Dynamic Light Scattering (DLS) using a Malvern Zetasizer nano ZS (Malvern Instruments Ltd., Worcestershire, UK). Measurements were conducted at 25 °C by transferring 1 mL of stock solution to a square cuvette for DLS analysis. A 50 mW laser with 638.2 nm wavelength was used as light source. Measurements were recorded at a detection angle of 173° (backscatter). The same measurements were also repeated after 1 h, in order to detect any agglomerates in FSW. NPs ζ-potential was measured using a Malvern Zetasizer nano ZS (Malvern Instruments Ltd., Worcestershire, UK). Measurements were conducted at 25 °C by transferring 1 mL of stock solution to a square cuvette for ζ-potential measurements.

2.2. Samples

Mature specimens of *P. lividus* were obtained from the hatchery in Camogli (CNR, Genoa, Italy), where they were maintained in ultrafiltered seawater (FSW). Adult samples were brought to the laboratory following the method suggested by Amemiya (1996), i.e. in a refrigerated bag, wrapped in tissues wet with sea water.

In this study, a spermiotoxicity test was performed. Therefore, sperms were exposed to NPs and the outcome developmental stages were analyzed. For brevity purposes, we shall refer to the larvae obtained from eggs fertilized by sperm exposed to the NPs as "exposed samples" and refer to sperm exposure with the words "treatment or "exposure".

2.3. Spermiotoxicity test

Spawning of gametes was obtained by oral administration of 1:1000 acetylcholine in FSW to avoid stress or toxicity associated with other current procedures. Eggs were collected in standard FSW, while sperms were collected 'dry', directly from the genital pores and maintained in aliquots of 200 μl at $T = 4$ °C.

Sperm was mixed from 3 different specimens. Experiments were repeated 4 times during breeding season and were carried out in triplicate.

For the test, 10 μl of 'dry' sperm were exposed to SiO_2 NP suspensions at serial concentrations (0.0001, 0.001, 0.01, 0.1, 1, 5, 10, 50 mg/L) for 1 h, by adding 1 mL NP suspension to the eppendorf vials, as previously described in Gambardella et al. (2013). Since to date no environmental concentration in both freshwater and seawater is available either in bibliography or in official database for SiO_2, test concentrations were chosen on the basis of ecotoxicological results of freshwater and marine species exposed to SiO_2 NPs, that tested concentrations up to 100 mg/L (Adams